

PAIN

## Expression of the nociceptin precursor and nociceptin receptor is modulated in cancer and septic patients

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### Editor's key points

- The nociceptin receptor has a role in pain and inflammatory pathways.
- Cells from patients with cancer pain or sepsis had higher nociceptin and lower pre-nociceptin mRNA expression than controls.
- There was no association with pain scores or analgesia, but some association with inflammatory markers.
- Further studies are needed.

**Background.** A role of nociceptin and its receptor (NOP) in pain and immune function has been suggested. The hypothesis was that mRNA expression of NOP and the nociceptin precursor pre-nociceptin (pN/OFQ) in peripheral blood cells differs in end-stage cancer patients suffering from chronic pain and septic intensive care unit (ICU) patients compared with healthy controls.

**Methods.** Blood samples were drawn from end-stage cancer patients and septic ICU patients. Additionally, postoperative patients representing individuals with surgical stress and healthy controls were enrolled as comparative groups. NOP and pN/OFQ mRNA expression, quantified by real-time polymerase chain reaction (RT-PCR), was compared between study groups, and associated to opioid medication, pain intensities, and the inflammatory markers procalcitonin (PCT) and interleukin-6.

**Results.** NOP expression was significantly higher in cancer patients [normalized ratio, median (inter-quartile range): 10.2 (7.4/17.8)], postoperative patients [8.0 (5.3/10.2)], and ICU patients [6.6 (4.2/9.5)] compared with healthy controls [4.4 (2.7/7.0);  $P < 0.001$ ]. Expression of pN/OFQ was lower in cancer patients [3.8 (1.9/5.9)] and ICU patients [1.9 (1.0/2.7)] but not in postoperative patients compared with healthy controls [7.2 (6.1/9.4);  $P < 0.001$ ]. Increased plasma PCT was associated with decreased pN/OFQ in all patient groups. In cancer patients, no association was seen with pain scores, opioid medication or duration of analgesia, and NOP or pN/OFQ mRNA.

**Conclusions.** NOP and pN/OFQ expression in peripheral blood cells was modulated in end-stage cancer and septic patients compared with healthy controls, whereas changes in postoperative patients were minor. The involvement of the NOP–pN/OFQ system in inflammation, impaired immune function, and pain has to be further elucidated.

**Keywords:** cancer; inflammation; opioid peptides, nociceptin-orphanin FQ; receptor, nociceptin

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The nociceptin receptor (NOP, opioid receptor-like 1, orphan opioid receptor, OP<sub>4</sub>) is a member of the G-protein-coupled receptor superfamily and exhibits about 60% sequence homology with the classical opioid receptors, especially in the putative membrane-spanning domains and intracellular loops.<sup>1–2</sup> Its endogenous agonist is N/OFQ (nociceptin, orphanin FQ).<sup>3–4</sup> It also shares sequence homology with classical opioid peptides such as dynorphin, however, with very low potency in binding to the classical opioid receptors and a distinct pharmacological profile.<sup>5–6</sup> The

cellular signal transduction pathway initiated by NOP activation appears nearly identical to that of classical opioid receptors. However, N/OFQ mediates analgesia when given spinally and hyperalgesia when given intra-cerebro-ventricularly.<sup>3–4</sup>

As the NOP–N/OFQ-system is involved in pain pathways, it is considered a promising target of pharmacological research in analgesic therapy.<sup>7–8</sup> Specifically, in inflammatory and neuropathic pain, a functional role is discussed.<sup>9</sup> N/OFQ has been implicated in a wide range of further physiological and

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behavioural functions, for example, effects on locomotion, anxiety, fear, stress, tolerance, dependence, feeding, learning, and memory.<sup>8 10 11</sup> The fact that both receptor and endogenous agonist are expressed in the human central nervous system and in immune cells at similar levels indicates that it may act as an important mediator of both nervous and immune responses in humans with possible involvement in the brain-immune axis.<sup>12 13</sup> Whereas some information on the NOP–N/OFQ system in septic patients is already available,<sup>8</sup> studies on the expression of NOP–N/OFQ in patients with cancer pain are lacking. However, this might be a population of specific interest, since immunological and inflammatory changes are prevalent and most of the patients suffer from cancer pain.

The present study investigates mRNA expression encoding NOP and the precursor for its endogenous ligand prepronociceptin (pN/OFQ) in whole blood from different cohorts of patients. The hypothesis was tested that patients suffering from end-stage cancer and also septic patients from the intensive care unit (ICU) displaying severe systemic inflammation might have altered baseline expression of NOP and pN/OFQ in peripheral blood cells. Postoperative patients representing subjects with surgical stress and healthy controls were also studied as comparative groups.

## Methods

### Patients and controls

The study was approved by the University Ethics Committee and was conducted according to the principles of the Declaration of Helsinki. Either the participants or a legal custodian gave written informed consent. Exclusion criteria were lack of informed consent or age younger than 18 yr. In the cancer patient group, 113 patients with advanced cancer were enrolled. Pain scores at rest and movement were recorded at admission and regularly during the hospital stay by the patients using a numeric rating scale (NRS, 0 denotes no pain; 100 denotes worst pain imaginable). The ICU patients consisted of 18 critically ill patients suffering from severe systemic inflammation and meeting the severe sepsis criteria. In cancer and ICU patients, blood samples (2.5 ml, PAXgene Blood RNA Tube, Qiagen/Becton Dickinson, Germany) were drawn on the day of routine blood sampling at admission. In ICU patients, additional blood was sampled on days 3 and 5 and also after recovery from inflammation-related organ dysfunction or at deterioration in the case of non-survivors.

Postoperative patients and healthy controls served as controls. The postoperative patient group ( $n=20$ ) had major elective surgery (nephrectomy, prostatectomy, bowel resection, ASA classification I–III) under general anaesthesia using a standardized institutional protocol (fentanyl 0.15 mg, propofol 2–3 mg kg<sup>-1</sup>, and cisatracurium for induction, remifentanyl/isoflurane-based maintenance of anaesthesia). Their blood was drawn after operation in the recovery room after emergence from anaesthesia. The healthy controls consisted of 28 healthy volunteers not taking any continuous medication.

### cDNA preparation and quantitative real-time polymerase chain reaction

RNA isolation from blood collected in PAXGene RNA tubes was performed according to the manufacturer's instructions (Pre-AnalytiX; Qiagen, Hilden, Germany). Total RNA was extracted using QIAamp<sup>®</sup> RNA Blood Kit (Qiagen, Hilden, Germany) and then dissolved in diethylpyrocarbonate-treated water and stored at –70°C until further analysis. cDNA was produced as polymerase chain reaction (PCR) template using 1st Strand cDNA Synthesis Kit for RT-PCR<sup>®</sup> [avian myeloblastosis virus (AMV+), Roche Diagnostics, Mannheim, Germany].

The PCRs for NOP, pN/OFQ, and the house-keeping gene human hypoxanthine phosphoribosyl-transferase (HPRT) were conducted in separate capillaries in duplicate on a Light-Cycler<sup>®</sup> 2.0 (Roche Diagnostics). The calibrator used was cDNA (1:50 diluted) prepared from the SK-N-DZ cell line. For the amplification of NOP, 4 µl of LightCycler<sup>®</sup> FastStart DNA Master<sup>PLUS</sup> SYBR Green I containing FastStart Taq DNA Polymerase, reaction buffer, dNTP mix, SYBR Green I dye, and MgCl<sub>2</sub> was used. Ten picomoles of each primer (NOP-f: 5'-TgCCgTTCTggg AggTTATCTA-3' and NOP-r: 5'-TTAgggTgAAggTgCTggTgA-3'; pN/OFQ-primers as published previously<sup>14</sup>) and 500 ng of prediluted cDNA (according to 30 ng total RNA) were added to the reaction mixture with a final volume of 20 µl. After an initial denaturation step at 95°C, amplification was performed with 45/40 cycles (denaturation 95°C for 5 s, annealing 60°C for 10 s, extension 72°C for 20 s).

For detection of HPRT, prediluted cDNA 500 ng, Universal ProbeLibrary 16 nM, Probe #73, LightCycler<sup>®</sup> TaqMan<sup>®</sup> Master 4 µl and 4 pmol of each primer (5'-TgACCTTgATTATTTTg CATACC-3' and 5'-CgAgCAAgACgTTCAGTCCT-3') was used. Fluorescence was monitored at the end of each cycle and detected in channel F2/F1. Data from RT-PCR, including calibrator and samples, were imported into the Relative Quantification Software and analysed with the Fit Coefficients File. This was created by PCR amplification of NOP and HPRT in a series of diluted cDNA (relative standard curve) in triplicates. The calculated normalized ratios (arbitrary units) reflect the expression level of target mRNA.

### Markers of inflammation

Procalcitonin (PCT) was measured by BRAHMS PCT sensitive Kryptor<sup>®</sup> test (Brahms GmbH, Hennigsdorf, Germany; normal values or insignificant systemic inflammatory response: PCT ≤ 0.5 ng ml<sup>-1</sup>; moderate systemic inflammatory response, e.g. SIRS: 0.5 < PCT ≤ 2.0 ng ml<sup>-1</sup>; severe systemic inflammatory response, sepsis: PCT > 2.0 ng ml<sup>-1</sup>). To determine interleukin-6 (IL-6) concentrations, the IL-6-ELISA-CB Kit (BioSource Europe S.A, Belgium) and the ELISA-Reader (BioRad Lab., USA) were used (normal values < 24 pg ml<sup>-1</sup>). White blood cell counts (leucocytes: normal range 4.0–10.0 × 10<sup>3</sup> µl<sup>-1</sup>; lymphocytes 18–45%, neutrophils 45–76%, monocytes 2–10%) and C-reactive protein (CRP, normal value < 5 mg litre<sup>-1</sup>) were quantified by standard clinical biochemical methods in the central hospital laboratory.

## Statistical analysis

Patient characteristic and standard laboratory data were expressed as mean (sd). Owing to high variance and no normal distribution of measures of mRNA expression, non-parametric comparison was performed using the Kruskal–Wallis test. A *P*-value of <0.05 was considered as statistically significant after correction for multiple testing. Results of relative quantification of mRNA are presented as medians (inter-quartile range, IQR). For correlation between mRNA expression and PCT, patients were clustered according to the designated manufacturer's PCT cut-off levels.

## Results

### Patients' characteristics

Patient characteristic and laboratory measures are displayed in Table 1 and details of the cancer patients in Table 2. The 18 ICU patients suffered from severe sepsis and nine of them died from sepsis-induced organ failure. From septic patients, a total of 53 blood samples were drawn. Postoperative subjects had comparable surgery-related data [ASA I–III, elective major abdominal or urological surgery, duration of anaesthesia: 202.3 (13.1) min, no signs of infection]. Healthy controls were aged 26–79 yr, which was comparable with the ICU group, but, about 10 yr younger than cancer and postoperative patients. Their medical history did not indicate any severe disease, and they were either taking no drugs at all or at least no drugs for severe systemic co-morbidity.

### Markers of inflammation

Laboratory markers of inflammation in ICU patients were elevated in accordance with the severity of their disease (Table 1). In cancer patients, a clinically relevant increase in inflammatory markers was observed in some patients, specifically in those with short survival. Correspondingly, IL-6 concentrations were increased (Table 3). PCT as sensitive marker of systemic inflammation due to infection was elevated in

63.9%, 36.0%, and 29.6% of the patients with end of life within 10, 10–30 days, or after 30 days, respectively.

### NOP and pN/OFQ expression in peripheral blood

Expression of NOP differed between patient groups and the healthy controls, with highest measures in cancer patients (Fig. 1A). NOP expression in ICU patients at admission was also increased. This change was mainly related to an increase in septic non-survivors [8.3 (5.5/10.1); *P*<0.001], whereas there was no difference in septic survivors [5.0 (4.0/8.2)] compared with healthy controls (HC). Sequential blood samples in ICU patients did not change over time. For pN/OFQ, expression was significantly lower in ICU and cancer patients (*P*<0.001) but not in postoperative patients (Fig. 1B).

Time of survival, daily opioid doses (morphine equivalents), and duration of opioid pretreatment (ranging between 0 and 72 weeks) were not related to NOP or pN/OFQ mRNA in cancer patients. The 16 cancer patients who were not taking any opioid medication did not differ in NOP [8.8 (7.5/15.9)] and pN/OFQ expression [3.4 (2.4/5.9)] compared with those receiving opioids [NOP: 10.9 (7.0/17.4); pN/OFQ: 3.7 (1.9/5.6)]. In addition, no difference could be observed in patients with no pain at rest (NRS=0; *n*=29), light pain (NRS=0–40; *n*=55), or severe pain (NRS≥45; *n*=29) at admission to the palliative care unit for NOP or pN/OFQ. Furthermore, there was no difference in expression between different kinds of cancer, existence of bone metastases, or liver metastases [NOP: 11.3 (7.5/23.5) vs 10.9 (7.8/16.9); pN/OFQ: 3.2 (1.4/5.0) vs 3.9 (2.0/6.3)].

For NOP, no significant correlation to plasma concentrations of PCT and IL-6 was seen. In contrast, patients with increased PCT had lower pN/OFQ mRNA expression compared with subjects with normal PCT values (*P*<0.001) (Fig. 2). These results were also verified in the subgroup of cancer patients [PCT≤0.5 ng ml<sup>-1</sup>: 4.3 (3.3/6.1), 0.5<PCT≤2.0 ng ml<sup>-1</sup>: 3.2 (2.1/6.9), PCT>2 ng ml<sup>-1</sup>: 1.9 (1.0/3.8); *P*<0.001].

**Table 1** Patient characteristic and laboratory data from patients suffering from cancer pain, patients with sepsis, postoperative patients, and healthy controls. Data are presented as number of patients, mean (sd), or median (IQR). Laboratory results deviating from the normal range are printed in bold. IL-6, interleukin-6; PCT, procalcitonin; n.m., not measured; ICU, intensive care unit

	Cancer patients	Septic ICU patients	Postoperative patients	Healthy controls
Number of patients	113	18	20	28
Male/female	53/60	12/6	10/8	18/9
Age (yr)	63.1 (13.7)	54.7 (13.9)	62.8 (12.2)	53.4 (15.6)
Leucocytes (×10 <sup>3</sup> m <sup>-3</sup> )	11.7 (10.3)	<b>14.5 (12.0)</b>	n.m.	6.7 (0.5)
Lymphocytes (%)	10.9 (7.7)	<b>6.9 (4.6)</b>	n.m.	n.m.
Neutrophils (%)	n.m.	<b>82.1 (13.8)</b>	n.m.	n.m.
Monocytes (%)	n.m.	<b>5.3 (3.8)</b>	n.m.	n.m.
IL-6 (pg ml <sup>-1</sup> )	<b>71.2 (116.1)</b>	<b>163.6 (108.4)</b>	<b>114.7 (154.0)</b>	n.m.
	24.0 (8.0/64.0)	<b>179 (50.4/249.0)</b>	<b>43.4 (23.2/134.8)</b>	
PCT (ng ml <sup>-1</sup> )	<b>3.1 (20.0)</b>	<b>38.3 (74.6)</b>	0.3 (0.2)	0.05 (0.02)
	0.2 (0.1/0.8)	<b>19.5 (1.4/25.2)</b>	0.3 (0.2/1.0)	

**Table 2** Patient characteristic and cancer-related details of cancer patients. Number (%) of patients or mean (SD)

Type of cancer	
Gastrointestinal tract	27 (23.9%)
Lung	23 (20.3%)
Breast	17 (15.0%)
Cervix, ovary	15 (13.3%)
Prostatic	11 (9.7%)
Kidney, bladder	10 (8.9%)
Others (oropharynx, liver, pancreas)	10 (8.9%)
Metastasis known	
Bone	43 (38.1%)
Liver	44 (38.9%)
Lung	21 (18.6%)
Visceral	31 (27.4%)
Kind of pain	
Bone	8 (7.1%)
Visceral	33 (29.2%)
Neuropathic	18 (15.9%)
Mixed	36 (31.9%)
Other symptoms	
Nausea/vomiting	37 (32.7%)/19 (16.8%)
Tiredness, fatigue/sedation	40 (35.4%)/9 (7.9%)
Constipation	50 (44.2%)
Cachexia	35 (30.7%)
Oedema	33 (29.2%)
Dyspnoea at exertion/at rest	32 (28.3%)/16 (14.2%)
Treatment	
Surgery	104 (92.0%)
Chemotherapy	74 (65.5%)
Radiation	55 (48.7%)
Analgesic drugs at admission no/WHO I/II/III	7 (6.2%)/9 (8.0%)/13 (11.5%)/84 (74.3%)
Co-analgesics: antidepressive/gabapentin or pregabalin	35 (31.0%)/5 (4.4%)
Cortison	49 (43.4%)
Duration of opioid treatment (months)	7.0 (15.8)
Patients with pain at rest/movement at admission	84 (74.3%)/99 (87.63%)

pN/OFQ expression also varied depending on IL-6 measures. pN/OFQ expression was 4.1 (2.2/6.1) for individuals with IL-6  $\leq 24$  pg ml<sup>-1</sup>, 2.5 (1.0/4.0) for those with IL-6 concentrations of 24–100 pg ml<sup>-1</sup>, and 1.4 (0.7/2.4) for patients with IL-6  $> 100$  pg ml<sup>-1</sup> ( $P < 0.001$ ).

## Discussion

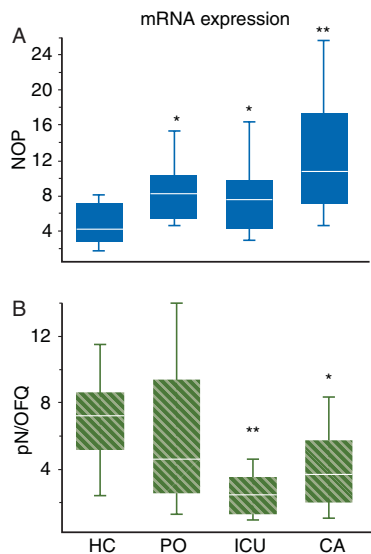
The present investigation shows that NOP and pN/OFQ mRNA expression are altered in severely ill patients compared with healthy controls, with highest NOP levels in cancer patients

**Table 3** Comparison of cancer patients allocated to different PCT groups. Data are presented as number of patients, mean (SD), or median (IQR). Laboratory results deviating from normal range are printed in bold. NRS, numeric rating scale 0–10 for pain scores; CRP, C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin; NRS, ANOVA, or the Kruskal–Wallis test where applicable;  $P$ -values refer to *post hoc* analysis; \*\* $P \leq 0.001$ ; \* $P < 0.05$

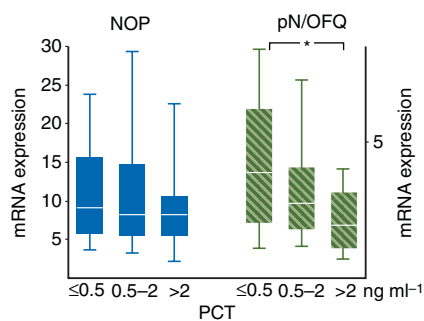
	PCT $\leq 0.5$ ng ml <sup>-1</sup>	0.5 ng ml <sup>-1</sup> <PCT $\leq 2.0$ ng ml <sup>-1</sup>	PCT $> 2.0$ ng ml <sup>-1</sup>
Number of patients (male/female)	77 (35/42)	23 (13/10)	13 (5/8)
Age (yr)	65.4 (7.6)	65.3 (11.9)	69.3 (8.0)
Survival (days)	70.2 (110.8)	38.0 (107.2)	18.9 (25.3)
Pain scores at admission (NRS)	20 (0/40)	0 (0/50)	25 (0/40)
rest/movement	70 (40/80)	75 (0/90)	45 (30/70)
Morphine equivalents/day (mg)	120 (40/180)	30 (0/90)	30 (20/120)
NOP (normalized ratio)	10.7 (7.3/16.4)	12.1 (7.4/24.5)	8.5 (5.9/23.1)
pN/OFQ (normalized ratio)	4.2 (2.4/6.3)	2.9 (2.1/4.5)	1.4 (0.9/2.6)*
Leucocytes ( $\times 10^3$ m <sup>-3</sup> )	9.9 (11.4)	<b>13.1</b> (7.9)	<b>24.1</b> (19.4)*
Lymphocytes (%)	<b>14.5</b> (9.8)	<b>7.9</b> (5.4)*	<b>7.5</b> (6.8)*
CRP (mg litre <sup>-1</sup> )	<b>8.7</b> (7.6)	<b>19.0</b> (22.6)*	<b>25.3</b> (17.2)**
IL-6 (pg ml <sup>-1</sup> )	<b>26.4</b> (37.2)	<b>78.0</b> (74.2)**	<b>224.6</b> (242.1)**
	14.2 (5.9/29.0)	47.3 (27.0/100.0)	157.8 (24.0/440.6)
PCT (ng ml <sup>-1</sup> )	0.15 (0.13)	<b>1.0</b> (0.04)**	<b>19.0</b> (49.8)**
	0.1 (0.07/0.17)	<b>0.8</b> (0.7/1.2)	<b>3.7</b> (2.7/4.5)

and lowest pN/OFQ in ICU patients. Although in the present study, there were varying proportions of cell types in patients' peripheral blood, specifically in the late stage cancer and sepsis, the relative quantification of mRNA content corrects for sample-to-sample variations caused by differences in quality and quantity of nucleic acid. A limitation of our study is the lack of measurement of protein which will be addressed in a future project. However, it is well accepted that peripheral blood mononuclear cells transcribe mRNA encoding NOP.<sup>15 16</sup> The receptor protein was detected on the cell surface of all types of white blood cells without any age- or sex-dependent differences.<sup>17</sup> The existence of NOP on T-cells and the ability of immune cells to produce N/OFQ<sup>5 14</sup> suggest an immunomodulatory role. For pN/OFQ, expression in peripheral blood mononuclear cells and





**Fig 1** mRNA expression of NOP and pN/OFQ (normalized ratio) in blood cells from healthy controls (HC), septic patients from the ICU, cancer patients (CA), and postoperative patients (PO). Box plots show medians (horizontal lines), IQR (boxes), and 5/95th percentile (error bars). \* $P < 0.05$ ; \*\* $P < 0.001$ . (A) Kruskal–Wallis test  $P < 0.001$ ; *post hoc* analysis PO vs HC:  $P = 0.01$ ; ICU vs HC:  $P = 0.01$ ; CA vs HC:  $P < 0.001$ . (B) Kruskal–Wallis test  $P < 0.001$ ; *post hoc* analysis PO vs HC:  $P = 0.2$ ; ICU vs HC:  $P < 0.001$ ; CA vs HC:  $P = 0.005$ .



**Fig 2** mRNA expression of NOP and pN/OFQ in all participants of the study. For septic patients, multiple blood samples during the course of sepsis were considered. Box plots show medians (horizontal lines), IQR (boxes), and 5/95th percentile (error bars). Kruskal–Wallis test \* $P < 0.001$ , *post hoc* analysis  $P < 0.01$  for patients with PCT (procalcitonin)  $\leq 0.5$  ng ml<sup>-1</sup> vs Group  $0.5 < \text{PCT} \leq 2$  ng ml<sup>-1</sup>; \*\* $P < 0.001$  for Group  $\text{PCT} \leq 0.5$  ng ml<sup>-1</sup> vs  $\text{PCT} > 2.0$  ng ml<sup>-1</sup>.

Clinical reports have mainly focused on nociceptin protein concentrations, however, no clear-cut correlation between circulating protein levels and pain states could be established in the past.<sup>19</sup> Whereas some groups reported increased plasma concentrations in pain states, for example, acute, sub-acute, and chronic pain compared with controls, other publications stated no change in women suffering from labour pain or decreased plasma nociceptin in fibromyalgia syndrome, cluster headache, and migraine.<sup>20–22</sup> In another trial, cerebrospinal fluid concentrations of nociceptin were linked to opioid medication with lowest levels in the morphine-treated group and highest in the untreated chronic pain group.<sup>23</sup>

Our study did not reveal an association of daily morphine equivalents or duration of opioid treatment and pain scores, type of cancer, or metastasis in cancer patients with either NOP or pN/OFQ expression. However, low number of patients within the subgroups and the heterogeneity of included cancer diseases may contribute to this lack of association. Furthermore, low peptide levels in plasma do not automatically mean that expression of encoding mRNA level is low. Nociceptin might also be released from other sources due to its wide prevalence in multiple organs.<sup>8</sup> Furthermore, it has to be considered that pN/OFQ is not only the precursor for nociceptin, but also for nocistatin, which reverses N/OFQ effects, but at a site other than NOP.<sup>24</sup>

### NOP–pN/OFQ and inflammation

There is ample evidence that opioid receptors and the endogenous opioid peptides underlie neuroimmune regulation responding to peripheral infectious stimuli.<sup>25</sup> The NOP–N/OFQ-system also appears to be integrated in such a neuroimmunomodulatory pathway. N/OFQ significantly contributes to endogenous pain control during prolonged nociceptive stimulation.<sup>26</sup> Homozygous NOP knockout mice or mice deficient for the N/OFQ precursor experience increased inflammatory hyperalgesia compared with wild-type animals. It is supposed that a prolonged nociceptive input is required to release N/OFQ. The lack of increase in pN/OFQ expression in postoperative patients might confirm these findings. Later time points of blood sampling might be more appropriate to detect any changes in mRNA expression. Activation of spinal NOP prevented acute cutaneous neurogenic inflammation in another trial and an endogenous N/OFQ-mediated decrease in pain hypersensitivity during inflammation was discussed.<sup>27</sup> Further experimental trials revealed divergent results in NOP mRNA expression after an immunological challenge with bacterial superantigen staphylococcal enterotoxin A (SEA).<sup>10</sup> Whereas the amount of splenic mRNA for NOP and N/OFQ was significantly reduced after SEA challenge in mice, there was the opposite trend in the thymus. Acosta and Davies<sup>28</sup> reported that LPS was a potent inducer of N/OFQ in sensory neurones, involving LPS receptor components of Toll-like receptor 4 and myeloid differentiation protein-1. However, neurones rather than blood cells were investigated. Williams

neutrophils has also been described.<sup>14 16</sup> *Ex vivo* studies confirmed that stimulated human polymorphonuclear neutrophils rapidly secrete N/OFQ by exocytosis with dose-dependent induction of polymorphonuclear chemotaxis.<sup>5 18</sup>

and colleagues<sup>14–29</sup> observed higher nociceptin protein measures in four septic non-survivors than in survivors. However, the measured values were low and no control group was included.

The present results add further evidence to the previously published hypothesis that there is a considerable modification of the NOP–N/OFQ-system in inflammatory states with significantly decreased pN/OFQ in patients with elevated PCT and IL-6. Previous data demonstrated that in contrast to haematological malignancies, solid carcinomas *per se* did not increase circulating PCT concentrations, regardless of the histotype or stage of the disease.<sup>30</sup> Increased PCT concentrations in the terminal phase of a malignancy are discussed as a result of local release of inflammatory mediators from the necrotizing tumour.<sup>31</sup> In addition to bacterial toxins, some cytokines like tumour necrosis factor (TNF)- $\alpha$  and IL-6 are able to trigger PCT synthesis.<sup>31</sup> It has to be emphasized that some of the cancer patients might have suffered from a concomitant infection, for example, pneumonia, urological tract infection, resulting in elevated markers of inflammation. However, only one patient was diagnosed with infection.

In contrast, modification of NOP and pN/OFQ expression was less pronounced in postoperative patients. It might be speculated that the perioperative stress response is a less potent stimulus for the NOP–N/OFQ system than sepsis or inflammatory and immunomodulatory changes in cancer patients. Furthermore, induction of cytokine and chemokine responses might take longer and were not detected in the blood sampled immediately after emergence from anaesthesia.

In the light of increasing interest in the clinical use of NOP as a target for pharmacotherapy in pain states and other diseases, our knowledge on the NOP–N/OFQ-pathway is still incomplete and further studies are necessary to define its regulation and the impact of application of exogenous NOP ligands.

## Conclusions

The present study underlines the modulation of the NOP–N/OFQ-pathway in end-stage cancer and patients with sepsis. The results emphasize a possible role of inflammatory and immunosuppressive processes, whereas pain intensities and opioid medication were not associated with the expression in peripheral blood cells of these end-stage cancer patients. Further details of the physiological and pathophysiological response of the NOP–N/OFQ system need to be elucidated in future patients' studies.

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## Conflict of interest

None declared.

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## References

- Calo' G, Guerrini R, Rizzi A, Salvadori S, Regoli D. Pharmacology of nociceptin and its receptor: a novel therapeutic target. *Br J Pharmacol* 2000; **129**: 1261–83
- Mollereau C, Parmentier M, Mailleux P, et al. ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. *FEBS Lett* 1994; **341**: 33–8
- Meunier JC, Mollereau C, Toll L, et al. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. *Nature* 1995; **377**: 532–5
- Reinscheid RK, Nothacker HP, Bourson A, et al. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. *Science* 1995; **270**: 792–4
- Fiset ME, Gilbert C, Poubelle PE, Pouliot M. Human neutrophils as a source of nociceptin: a novel link between pain and inflammation. *Biochemistry* 2003; **42**: 10498–505
- Taylor F, Dickenson A. Nociceptin/orphanin FQ. A new opioid, a new analgesic? *Neuroreport* 1998; **9**: R65–70
- Zeilhofer HU, Calo G. Nociceptin/orphanin FQ and its receptor—potential targets for pain therapy? *J Pharmacol Exp Ther* 2003; **306**: 423–9
- Lambert DG. The nociceptin/orphanin FQ receptor: a target with broad therapeutic potential. *Nat Rev Drug Discov* 2008; **7**: 694–710
- Chen Y, Sommer C. Nociceptin and its receptor in rat dorsal root ganglion neurons in neuropathic and inflammatory pain models: implications on pain processing. *J Peripher Nerv Syst* 2006; **11**: 232–40
- Goldfarb Y, Reinscheid RK, Kusnecov AW. Orphanin FQ/nociceptin interactions with the immune system in vivo: gene expression changes in lymphoid organs and regulation of the cytokine response to staphylococcal enterotoxin A. *J Neuroimmunol* 2006; **176**: 76–85
- Mogil JS, Pasternak GW. The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol Rev* 2001; **53**: 381–415
- Peluso J, LaForge KS, Matthes HW, Kreek MJ, Kieffer BL, Gaveriaux-Ruff C. Distribution of nociceptin/orphanin FQ receptor transcript in human central nervous system and immune cells. *J Neuroimmunol* 1998; **81**: 184–92
- Wick MJ, Minnerath SR, Roy S, Ramakrishnan S, Loh HH. Expression of alternate forms of brain opioid 'orphan' receptor mRNA in activated human peripheral blood lymphocytes and lymphocytic cell lines. *Brain Res Mol Brain Res* 1995; **32**: 342–7
- Williams JP, Thompson JP, Rowbotham DJ, Lambert DG. Human peripheral blood mononuclear cells produce pre-pro-nociceptin/orphanin FQ mRNA. *Anesth Analg* 2008; **106**: 865–6
- Williams JP, Thompson JP, McDonald J, et al. Human peripheral blood mononuclear cells express nociceptin/orphanin FQ, but not mu, delta, or kappa opioid receptors. *Anesth Analg* 2007; **105**: 998–1005

- 16 McDonald J, Leonard AD, Serrano-Gomez A, et al. Assessment of nociceptin/orphanin FQ and micro-opioid receptor mRNA in the human right atrium. *Br J Anaesth* 2010; **104**: 698–704
- 17 Kruger C, Kothe L, Struppert A, Pietruck C, Simm A, Grond S. Expression und function of the ORL-1 receptor on human leukocytes. *Schmerz* 2006; **20**: 509–18
- 18 Serhan CN, Fierro IM, Chiang N, Pouliot M. Cutting edge: nociceptin stimulates neutrophil chemotaxis and recruitment: inhibition by aspirin-triggered-15-epi-lipoxin A4. *J Immunol* 2001; **166**: 3650–4
- 19 Barnes TA, Lambert DG. Editorial III: Nociceptin/orphanin FQ peptide-receptor system: are we any nearer the clinic? *Br J Anaesth* 2004; **93**: 626–8
- 20 Anderberg UM, Liu Z, Berglund L, Nyberg F. Plasma levels on nociceptin in female fibromyalgia syndrome patients. *Z Rheumatol* 1998; **57**(Suppl 2): 77–80
- 21 Ertsey C, Hantos M, Bozsik G, Tekes K. Plasma nociceptin levels are reduced in migraine without aura. *Cephalalgia* 2005; **25**: 261–6
- 22 Ertsey C, Hantos M, Bozsik G, Tekes K. Circulating nociceptin levels during the cluster headache period. *Cephalalgia* 2004; **24**: 280–3
- 23 Raffaelli W, Samolsky Dekel BG, Landuzzi D, et al. Nociceptin levels in the cerebrospinal fluid of chronic pain patients with or without intrathecal administration of morphine. *J Pain Symptom Manage* 2006; **32**: 372–7
- 24 Johnson EE, Connor M. Towards a receptor for nocistatin? *Br J Pharmacol* 2007; **152**: 415–6
- 25 Rittner HL, Brack A, Stein C. Pain and the immune system. *Br J Anaesth* 2008; **101**: 40–4
- 26 Depner UB, Reinscheid RK, Takeshima H, Brune K, Zeilhofer HU. Normal sensitivity to acute pain, but increased inflammatory hyperalgesia in mice lacking the nociceptin precursor polypeptide or the nociceptin receptor. *Eur J Neurosci* 2003; **17**: 2381–7
- 27 Dong XW, Williams PA, Jia YP, Priestley T. Activation of spinal ORL-1 receptors prevents acute cutaneous neurogenic inflammation: role of nociceptin-induced suppression of primary afferent depolarization. *Pain* 2002; **96**: 309–18
- 28 Acosta C, Davies A. Bacterial lipopolysaccharide regulates nociceptin expression in sensory neurons. *J Neurosci Res* 2008; **86**: 1077–86
- 29 Williams JP, Thompson JP, Young SP, et al. Nociceptin and urotensin-II concentrations in critically ill patients with sepsis. *Br J Anaesth* 2008; **100**: 810–4
- 30 Giovanella L, Suriano S, Ricci R, Ravani P, Ceriani L. Circulating procalcitonin in aseptic carcinoma patients: a specificity study with (18)F-fluorodeoxyglucose positron-emission tomography/computed tomography as benchmark. *Clin Chem Lab Med* 2010; **48**: 1163–5
- 31 Gorisek B, Miksic NG, Krajnc P, Pakiz M, Turk Z. The role of procalcitonin in gynaecological surgery. *J Int Med Res* 2009; **37**: 918–26